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Chapter V

Renal function in cancer patients treated with hyperthermic isolated limb perfusion with recombinant tumor necrosis factor alpha and melphalan

In press as:

Renal function in cancer patients treated with hyperthermic isolated limb perfusion with recombinant tumor necrosis factor alpha and melphalan

Nephron

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Summary

Hyperthermic isolated limb perfusion (HILP) with recombinant TNF- α (r-TNF- α) and melphalan has been shown to result in a sepsis like syndrome due to leakage of r-TNF- α from the perfusion system to the systemic circulation. We have studied renal function parameters in 11 cancer patients, who underwent 12 perfusions. Three patients, perfused with melphalan only, served as controls. All patients treated with r-TNF- α developed a sepsis syndrome and needed volume replacement and inotropes to remain normotensive; controls had an uneventful postoperative course. Creatinine clearance decreased transiently on the day of perfusion in both groups (mean preperfusion clearance 118 ml/min, mean postperfusion clearance 68 ml/min, $p < 0.02$, $n = 15$). Follow-up measurements of renal plasma flow and glomerular filtration rate in 9 r-TNF- α treated patients did not suggest permanent damage. One patient became hypotensive and developed transient multiple organ dysfunction with renal failure needing hemofiltration. In r-TNF- α treated patients, but not in controls, a transient increase in clearance of β_2 microglobulin (49 vs. 8171 ml/min, $p < 0.001$) and urinary excretion of phosphate (12 vs. 48 mmol/L, $p < 0.05$) was seen, compatible with proximal tubular dysfunction. These data suggest that HILP with melphalan decreases glomerular function, whether or not r-TNF- α is added to the perfusion circuit. Extension of the treatment regimen with r-TNF- α may result in additional proximal tubular dysfunction. If hypotension can be avoided this deterioration in renal function seems to be transient, with full recovery within weeks.

Introduction

Impairment of renal function is a frequent complication of septic shock, with a major impact on outcome. Its occurrence is at least partly explained by a decrease in renal blood flow secondary to a drop in arterial blood pressure. Additionally, cytokines like tumor necrosis factor alpha (TNF- α), which are released systemically and locally during bacterial sepsis, may directly compromise renal function.

Recently, hyperthermic isolated limb perfusion with melphalan and human recombinant TNF- α (r-TNF- α) has been studied in patients with locally advanced soft tissue tumors and advanced melanomas of a limb, as an alternative to amputation. The technique of isolated limb perfusion allows the administration of high doses of these agents locally while minimizing systemic toxicity. However, it has been recognized by us as well as by others that isolated limb perfusion with r-TNF- α induces a sepsis like state in all patients, characterized by fever, tachycardia and a low blood pressure due to systemic vasodilation [1,2]. The occurrence of this syndrome is explained by leakage of r-TNF- α from the perfused limb into the systemic circulation; very high levels of TNF- α have been documented in peripheral blood of these patients during and directly following perfusion. Leakage has been confirmed by adding radiolabeled albumin to the perfusate; radioactivity can be traced to the systemic circulation during perfusion treatment [3].

In view of the similarity of this clinical syndrome to bacterial sepsis and the unexpectedly high systemic levels of TNF- α , a detrimental effect on kidney function could be anticipated. This study was undertaken to assess the impact of isolated limb perfusion with r-TNF- α and melphalan on creatinine clearance, renal plasma flow (ERPF) and glomerular filtration rate (GFR). Fractional excretion of sodium (FE_{Na}) and urinary excretion of β_2 microglobulin and phosphate were determined to assess proximal tubular function.

Patients and methods

Outline of Experiments

Creatinine clearance and excretion of β_2 microglobulin and phosphate were determined in patients undergoing hyperthermic isolated limb perfusion with r-TNF- α and melphalan. The results were compared with data obtained in control patients who underwent a similar perfusion procedure but without the addition of r-TNF- α . In a subgroup of r-TNF- α treated patients ERPF and GFR were determined with radiopharmaceuticals prior to the perfusion. These experiments were repeated after the perfusion. Due to background radioactivity from radio-labeled albumin administered during the perfusion, postoperative renal function measurement had to be postponed with an average of 22 days (range 10-31).

Anesthesia and Intensive Care

Anesthesia was induced with thiopental, after which the patients were paralyzed with vecuronium and the trachea intubated. Anesthesia was maintained with midazolam, sufentanyl, nitrous oxide and isoflurane. After induction arterial and pulmonary artery catheters were inserted. Blood pressure, ECG, urine output, venous and pulmonary pressures, as well as pulmonary wedge pressures and blood gas values were checked at standard intervals. Fluid resuscitation with crystalloid and colloid solutions was given to maintain pulmonary wedge pressures above 12 mmHg and a dopamine infusion was added if

systolic arterial blood pressure fell to 90 mmHg or decreased by > 30 mmHg from preoperative values despite adequate fluid replacement. All patients were admitted to the intensive care unit following surgery, where the same algorithm was followed to prevent hypotension. Patients received mechanical ventilation until hemodynamically stable.

Hyperthermic Isolated Limb Perfusion

The perfusion technique employed at the Groningen University Hospital is based on the technique developed by Creech and Kremenz [4]. Briefly, after ligation of all collateral vessels and heparinization of the patient with 3.3 mg heparin/kg bodyweight (Thromboliquine, Organon BV, Oss, the Netherlands) the axillary, iliac, femoral or popliteal vessels were dissected, cannulated and connected to the extracorporeal circuit. A tourniquet was applied to the proximal limb in an attempt to minimize leakage of the perfusate into the systemic circulation through skin collaterals. Perfusion was performed under mild hyperthermic conditions (39-40°C). The perfusate consisted of 350 ml 5% dextran 40 in glucose 5% (Isodex, Pharmacia AB, Uppsala, Sweden), 500 ml blood (250 ml red blood cells, 250 ml plasma), 30 ml 8.8% NaHCO₃ and 0.5 ml 5000 IU/ml heparin (Thromboliquine). The perfusate was oxygenated with a bubble oxygenator and driven by a roller pump. At the start of perfusion recombinant human TNF- α (Boehringer, Ingelheim, Germany, 4 mg for leg perfusions and 3 mg for arm perfusions) was injected as a bolus into the arterial line of the perfusion circuit. Melphalan (Burroughs Wellcome, London, England, 10 mg/L volume of an affected leg and 13 mg/L volume of an affected arm) was administered 30 minutes later. After 90 minutes of perfusion, the limb was flushed with 2 L dextran 40 in glucose 5% (Isodex) and 500 ml blood (250 ml red blood cells, 250 ml plasma), catheters were removed, the circulation was restored and the heparin antagonized with protamine chloride.

Measurement of TNF- α

TNF- α was measured in blood drawn from the radial artery by specific immunoradiometric assay (Medgenix Diagnostics, Soesterberg, the Netherlands). Samples were processed according to the guidelines of the manufacturer.

Measurement of renal function parameters

GFR and ERPF were measured simultaneously using ¹²⁵I-iothalamate and ¹³¹I-hippurate, respectively, according to the method described by Donker et al. [5]. The radiopharmaceuticals were infused at a constant rate after a priming dose was given. After an equilibration period of one and a half hour, clearances were determined over a two hour period. Post-operative testing was performed at an average of 22 days (SD 6 days) after perfusion. Creatinine clearances were determined from 24 hour urine collections.

Statistical Analysis

Data were analyzed using SPSS for MS WINDOWS (release 5.0). Differences in mean values within groups were studied with either a t-test for paired differences (systemic vascular resistance, creatinine clearance) or a Wilcoxon Matched Pairs Signed-Ranks Test (ERPF, GFR, urinary excretion of β_2 microglobulin, serum levels of β_2 microglobulin, clearance of β_2 microglobulin, urinary excretion of phosphate), depending on the assumption of distribution. Differences between groups were studied with either an independent samples t-test (creatinine clearance) or a Mann-Whitney U-Wilcoxon Rank Sum W Test (serum levels of β_2 microglobulin, urinary excretion of β_2 microglobulin and FE_{Na}).

Bonferroni's method was used to correct for the effect of multiple comparisons. For single testing a p-value <0.05 was considered significant.

Results

Between September 1992 and May 1993 twelve consecutive hyperthermic isolated limb perfusions with r-TNF- α and melphalan were studied in eleven patients. Three patients, perfused with melphalan but without r-TNF- α served as controls. Paired data on renal blood flow and glomerular filtration rate were available in seven and nine perfusions respectively.

Peak systemic TNF- α concentrations in patients treated with r-TNF- α ranged from 4328 ng/L to 267000 ng/L with a median of 16969 ng/L and a mean of 67693 ng/L. Maximum TNF- α concentrations were reached at the end of the perfusion procedure.

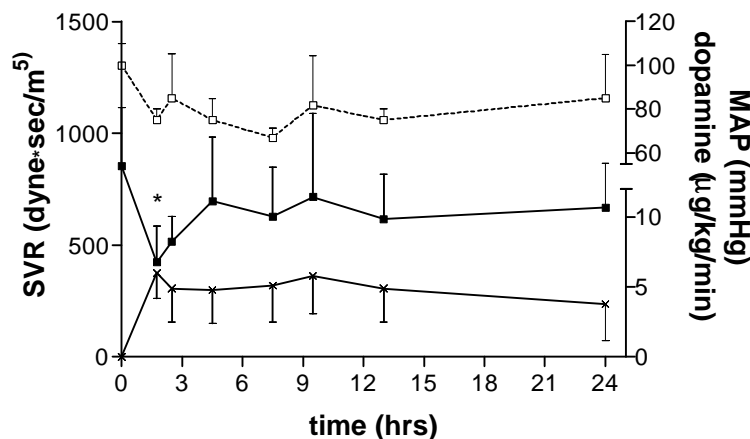


Fig. 1 Mean arterial pressure (MAP, open rectangles), systemic vascular resistance (SVR, closed rectangles) and dopamine requirement (crosses) in patients treated with r-TNF- α perfusion. The asterisk (*) illustrates a significant decrease in SVR compared to baseline values ($p<0.005$). Error bars represent standard deviations.

Figure 1 shows the effects of r-TNF- α perfusion on systemic vascular resistance, mean arterial pressure and the average need for dopamine. In the r-TNF- α -treated group mean systemic vascular resistance decreased from 854 dyne/sec/cm⁻⁶ preperfusion to a nadir of 423 dyne/sec/cm⁻⁶ postperfusion ($p<0.005$). Mean arterial pressure was not significantly decreased at any moment; the lowest mean arterial pressure recorded was 60 mm Hg. All but one r-TNF- α treated patient needed treatment with dopamine to maintain bloodpressure after adequate volume resuscitation (maximum dose 12 mg/kg/min). This patient, and 3 others, were treated with norepinephrine. None of the control patients needed postoperative care in the ICU and none of them were treated with dopamine and/or norepinephrine postoperatively.

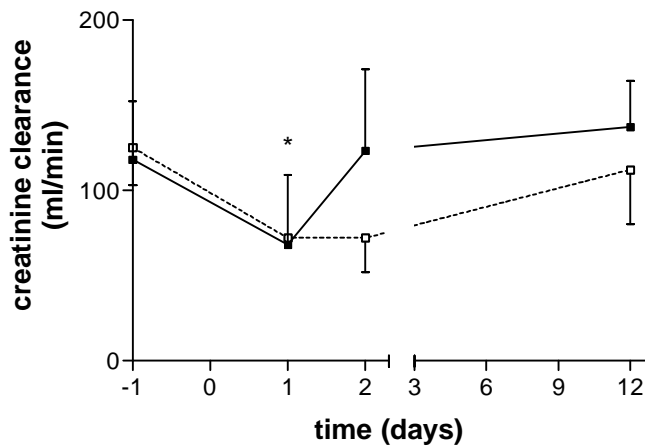


Fig. 2 Mean creatinine clearance in patients treated with r-TNF- α perfusion (solid line) and in controls (dotted line). The asterisk (*) illustrates a significant decrease in creatinine clearance in both groups compared to baseline values ($p<0.02$). Error bars represent standard deviations.

Preperfusion serum creatinine levels ranged from 67-90 $\mu\text{mol/L}$ (mean 80 $\mu\text{mol/L}$). All patients, including controls, showed a transient drop in creatinine clearance within 48 hours following perfusion (Figure 2). Mean creatinine clearance decreased from a preperfusion level of 118 ml/min to a nadir of 68 ml/min on the second day after the perfusion ($p<0.02$). There was no significant difference in creatinine clearance between r-TNF- α treated patients and controls. Creatinine clearance had returned to normal in all patients on day 4, except in one patient who maintained a septic hemodynamic pattern and developed multiple organ failure.

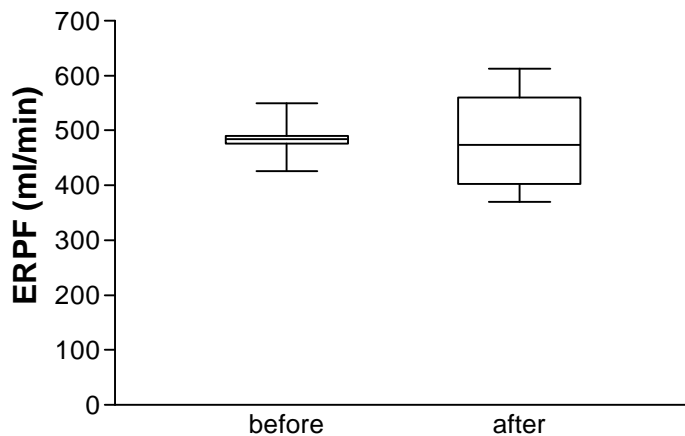


Fig. 3 Box and whiskers plot showing effective renal plasma flow (ERPF), standardized for body surface area, before and after perfusion with r-TNF- α .

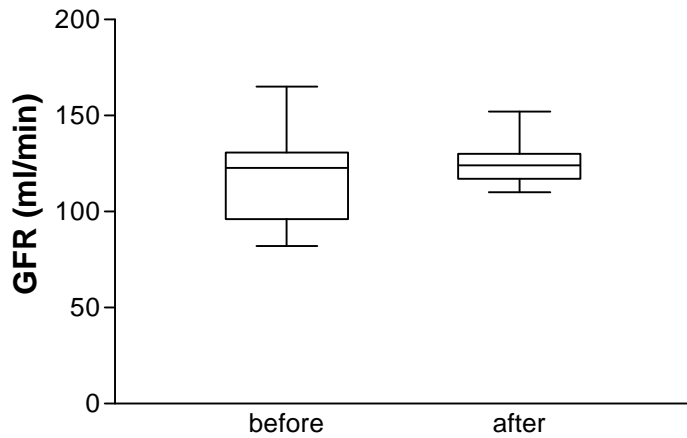


Fig. 4 Box and whiskers plot showing glomerular filtration rate (GFR), standardized for body surface area, before and after perfusion with r-TNF- α .

Figure 3 and 4 show pre- and postperfusion values in r-TNF- α treated patients for ERPF (n=7) and GFR (n=9) respectively; no significant differences were observed in any of these paired parameters.

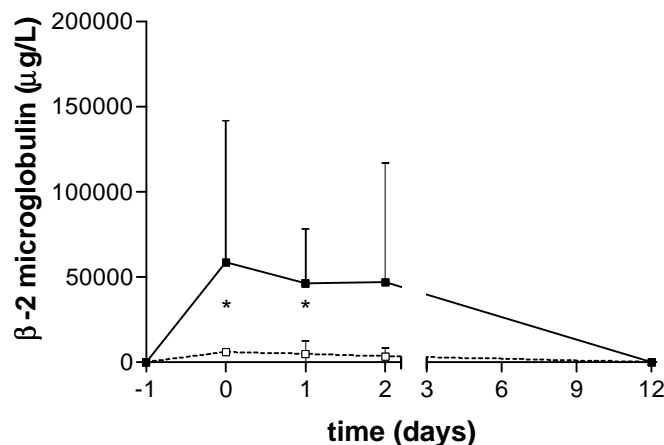


Fig. 5 Urinary excretion of β_2 microglobulin in patients treated with r-TNF- α perfusion (solid line) and in controls (dotted line). The asterisks (*) illustrate a significant increase in β_2 microglobulin excretion in the r-TNF- α -treated group compared to baseline values ($p<0.01$). Numbers represent median values.

In the r-TNF- α treated group there was an increase in the excretion of β_2 -microglobulin in the urine from a median value of 41 mg/24 h preoperatively to a median value of 36727 mg/24 h one day postperfusion ($p<0.01$, Figure 5). In serum, β_2 -microglobulin concentrations in this group rose from a mean preperfusion value of 1416 mg/L to a mean one day postperfusion value of 3060 mg/L ($p<0.01$, Figure 6).

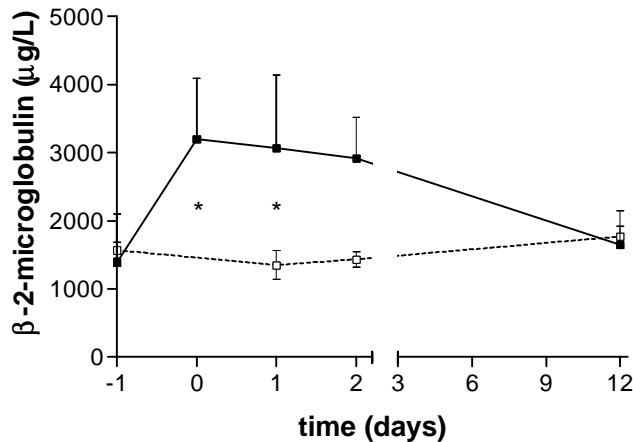


Fig. 6 Mean serum levels of β_2 microglobulin in patients treated with r-TNF- α perfusion (solid line) and in controls (dotted line). The asterisks (*) illustrate a significant increase in serum β_2 microglobulin concentrations in the r-TNF- α -treated group compared to baseline values ($p<0.01$). Error bars represent standard deviations.

Clearance of β_2 -microglobulin in patients perfused with r-TNF- α increased from 49 ml/min pre-operatively to 8171 ml/min one day postperfusion ($p<0.001$). In the control group neither urinary excretion of β_2 -microglobulin, nor serum concentrations of β_2 -microglobulin changed significantly over time (Fig 5 and 6). On the first day after perfusion, levels of β_2 -microglobulin in serum, as well as urinary excretion of β_2 -microglobulin, were significantly higher in the r-TNF- α treated patients than in controls ($p<0.05$). Preoperative values were not significantly different.

In r-TNF- α perfused patients urinary excretion of phosphate increased from a mean pre-operative value of 12 mmol/24 h to a mean value of 48 mmol/24 h on day 2 after perfusion ($p<0.05$). If corrected for sodium excretion this difference was no longer significant.

Fe_{Na} increased from a preperfusion level of 0.4% to 1.1% on day 1 after perfusion with r-TNF- α , but this increase failed to reach statistical significance ($p=0.07$).

Discussion

This study describes a group of cancer patients who were inadvertently exposed to high systemic concentrations of TNF- α and developed signs of sepsis syndrome as a result. Exposure to TNF- α occurred as a side effect of hyperthermic isolated limb perfusion with r-TNF- α , through leakage of this antitumor cytokine from the perfusion circuit to the systemic circulation. The mean maximum systemic TNF- α concentration in r-TNF- α treated patients was 67693 ng/L (range 4328 to 267000 ng/L). This is several orders of magnitude higher than TNF- α concentrations previously reported in different forms of shock. In a series of 79 patients with meningococcal disease and/or septicemia all patients with TNF- α levels over 100 ng/L died [6]. In septic shock median TNF- α concentrations of 120 ng/L have been reported [7]. Despite much higher concentrations of TNF- α all our patients survived their ICU stay and could be discharged to the ward after a median stay in the ICU of two days.

The effects of TNF- α on the kidney have not yet been well characterized. TNF- α is an early mediator of endotoxemic shock and, if administered to laboratory animals, causes a clinical syndrome

which is very similar to bacterial sepsis [8]. In this setting, part of the effect of TNF- α on the kidney will be through decreased mean arterial pressure and decreased renal perfusion. However, there may also be a direct effect of TNF- α on kidney function. Rabbits given recombinant human TNF- α showed a decrease in renal blood flow, but also a decrease in the renal fraction of aortic blood flow, caused by a 17% increase in renal resistance [9]. Autoregulation was preserved as indicated by a compensatory increase of the filtration fraction with 17%. Hemodynamic changes were abolished by both thromboxane inhibitors and indomethacin, implying a role for the arachidonate cascade system. In another rabbit model TNF- α induced swelling of glomerular endothelial cells and accumulation of leukocytes in the glomerular capillary lumen [10]. In a plethora of animals models, local TNF- α production, mainly by mesangial cells, has been shown to play a role in the pathophysiology of inflammatory kidney disease [11- 13]. TNF- α is also involved in kidney transplant rejection [14]. Experiments in humans have been limited by the toxicity of TNF- α . Early trials with intravenous TNF- α have reported renal toxicity in sporadic cases [15], but normal kidney function is the rule [16]. In patients who underwent isolation perfusion with r-TNF- α Eggimann and coworkers reported a fall in creatinine clearance in 22% of their patients [1]. In a group of 9 patients reported by Sorkin and coworkers, 2 patients showed a transient rise in serum creatinine [17].

In the present study all r-TNF- α treated patients showed a decrease in creatinine clearance on the day of perfusion. There was a similar decrease in control patients, who were treated with melphalan only. In both groups creatinine clearance returned to normal levels within two days. Subsequent radiopharmaceutical studies in the r-TNF- α treated group did not show significant late changes in GFR and ERPF. It should be noted that in all patients but one hypotension could be prevented with early expansion of volume and judicious use of vasocactive drugs, guided by invasive haemodynamic monitoring in the operating room as well as in the intensive care unit. One patient went on to develop transient multiple organ failure. This patient remained inotrope-dependent with a septic hemodynamic profile and developed acute respiratory failure and non-oliguric renal failure for which he had to be treated with continuous veno-venous hemofiltration.

It appears from these data that hyperthermic isolated limb perfusion with melphalan *as such* transiently decreases creatinine clearance. The role of r-TNF- α in this setting remains to be determined. We were unable to show that the addition of r-TNF- α to the perfusion circuit resulted in any additional loss of glomerular function. Peri- and postoperative hypotension, due to TNF- α -induced systemic vasodilation, can be lifethreatening, and its management requires skill and experience. Aggressive support of perfusion pressures with cristalloid and colloid infusions with vasopressors in an ICU setting is mandatory to prevent the development of multiple organ dysfunction. In the series described here, no permanent renal damage occurred if peri- and postoperative hypotension could be avoided. In theory, this could be attributed to a specific protective effect of dopamine on the kidney. However, proof that dopamine influences the course of acute renal failure is lacking. Furthermore, in additional experiments in mechanically ventilated patients after aortic surgery, we were unable to show a specific renal hemodynamic effect of this drug; the observed increase in RBF and GFR could be fully ascribed to the increase in cardiac output [18].

On the day of perfusion, excretion of β_2 microglobulin increased greatly in r-TNF- α treated patients. There was a simultaneous rise in plasma β_2 microglobulin, which has been recognized as a TNF- α effect by others [19], but this was insufficient to explain the 150-fold increase in clearance of β_2 microglobulin. Like the creatinine clearance, clearance of β_2 microglobulin returned to normal levels within two days. Since β_2 microglobulin is almost completely reabsorbed in the proximal tubule these data suggest a temporary dysfunction of this proximal part of the nephron. The observed increase in

phosphate excretion is compatible with such a temporary proximal dysfunction, although it could also be explained by the observed increase in sodium excretion. An increase in sodium excretion in turn, could be attributed to sodium loading, which was carried out to correct hypovolemia and to prevent hypotension. Although FE_{Na} was $>1\%$ in the r-TNF- α treated group on day 1 postperfusion, a value compatible with a diagnosis of acute tubular necrosis, it did not differ significantly from FE_{Na} in the control group. Thus, the precise effect of r-TNF- α on proximal tubular function remains to be determined.

The present study has several limitations. The control group of patients treated with perfusion with melphalan but without r-TNF- α was small and patients were not randomly assigned to either treatment arm. Due to its dramatic effects on tumor regression, perfusions without r-TNF- α became to be considered ethically unjustified by the responsible clinicians. Obviously, this made any form of randomization impossible. A second drawback of the study design is the interference of GFR and ERPF measurements with standard I^{131} -albumin measurement of albumin leak during perfusion (as an indicator of r-TNF- α leak to the systemic circulation), forcing us to postpone GFR and ERPF measurements to well after the perfusion procedure. In theory early changes in renal function may thus have been masked. However, to our knowledge this is the first study to examine the renal effects of isolated limb perfusion with r-TNF- α in humans in some detail.

We conclude that, in hyperthermic isolated limb perfusion with r-TNF- α and melphalan, renal toxicity is acceptable, even if considerable leakage of r-TNF- α to the systemic circulation of the patient cannot be avoided. Although a transient decrease in creatinine clearance is observed, no lasting damage to the kidney will occur if hemodynamic stability can be maintained. This requires early treatment with volume expansion and pressor drugs, guided by invasive haemodynamic monitoring. If r-TNF- α is added to the treatment a transient decrease of proximal tubular function is seen, in addition to the effect on glomerular filtration. This may represent a direct nephrotoxic effect of TNF- α .

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